INDEPENDENT LABORATORY VALIDATION OF METHOD NUMBER DUPONT-2392, "ANALYTICAL METHOD FOR THE DETERMINATION OF OXAMYL AND ITS OXIME METABOLITE IN SOIL USING LC/MS ANALYSIS

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1.0 SUMMARY

The purpose of this study is to conduct an independent laboratory validation (ILV) on analytical method DuPont-2392 "**Analytical Method for the Determination of Oxamyl and Its Oxime Metabolite in Soil Using LC/MS Analysis**". The ILV of analytical methods is required by the U.S. EPA (draft Ecological Effects Test Guidelines, OPPTS 850.7100, Data Reporting for Environmental Chemistry Methods).

2.0 INTRODUCTION

Vydate® insecticide/nematicide products are used to control insects, mites, and nematodes during the production of various field crops, fruits and vegetables. The active ingredient of Vydate® is oxamyl. In water, oxamyl undergoes hydrolysis to produce oxime. An analytical method was developed and validated for the detection and quantitative analysis of oxamyl and its oxime metabolite in soil. This method was used and was intended for support of a small-scale prospective groundwater monitoring study for oxamyl. The method developed and validated is DuPont-2392 "**Analytical Method for the Determination of Oxamyl and Its Oxime Metabolite in Soil Using LC/MS Analysis**". The purpose of this study is to independently validate DuPont-2392. The ILV of analytical methods is required by the U.S. EPA (draft Ecological Effects Test Guidelines, OPPTS 850.7100, Data Reporting for Environmental Chemistry Methods). Henceforth in this report, the oxime metabolite will be referred to as "oxime".

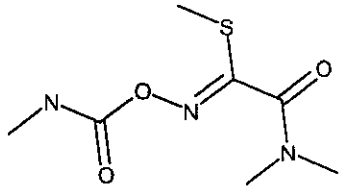
The soil selected for this ILV is representative of soil analyzed during the small-scale prospective groundwater monitoring study (References 1-2). The LOQ and lowest fortification level tested for oxamyl and oxime in soil using DuPont-2392 was 10.0 ng/g (ppb). During method ILV, the analytical method was tested at 1X and 10X the LOQ for oxamyl and oxime.

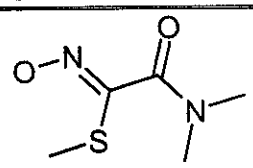
The analytical method involved the extraction of oxamyl and oxime from soil using an accelerated solvent extractor (ASE) system. An aliquot of the extract was removed. A 1-mL aliquot of aqueous formic acid was added, and the extracts were concentrated under a flow of nitrogen. The samples were then diluted to a 10-mL volume using a 0.01% aqueous formic acid solution. The samples were then syringe filtered and analyzed using LC/MS detection. The instrument used for sample analysis during the ILV was a triple quadrupole mass spectrometer operated in the LC/MS single ion mode. This analytical method passed ILV on the first attempt without any major modifications.

3.0 MATERIALS AND METHODS

3.1 Test Substances

Summaries of the test substances are provided in the tables below.

Common Name	Oxamyl
Structure	
DPX Number	DPX-D1410
CAS Chemical Name	Methyl 2-(dimethylamino)-N-[[(methylamino)carbonyl]oxy]-2-oxoethanimidothioate
CAS Registry Number	23135-22-0
Lot Number	376
Purity	100.0%
Storage Conditions	Room Temperature

Common Name	Oxime
Structure	
DPX Number	IN-A2213
CAS Chemical Name	Methyl 2-(dimethylamino)-N-hydroxy-2-oxoethanimidothioate
CAS Registry Number	66344-33-0
Lot Number	10
Purity	99.9%
Storage Conditions	Room Temperature

3.2 Test System

The analytical method was validated using soil provided by the Sponsor Representative. The soil provided was the same soil used in the Small Scale Prospective Groundwater Monitoring Study for Oxamyl (Reference 2). The soil was from an agricultural field in Edgecombe County, NC. Samples of surface soil were collected from the untreated control plot of the study AMR 4318-97. The soil is sandy (approximately 90% sand w/w), has an organic matter content of about 1% or less, and a soil pH of about 5.8. The average percent moisture content for the soil used in these validation analyses was determined to be 4.0% with a standard deviation of 0.4%.

3.3 Equipment

Instrumentation

Mass Spectrometer System: Micromass Quattro II LC/MS/MS with an electrospray (ESI) Interface (Micromass Inc., Beverly, MA)

LC system: HP1100 (Hewlett-Packard, Wilmington, DE)

Chromatographic Supplies

HPLC Column: 2.1 mm i.d. × 10 cm, Hewlett-Packard® Hypersil ODS column with 3-µm diameter packing, PN 79916ODS-352, (Hewlett-Packard, Wilmington, DE)

HPLC Vials, Target DP Amber Kit, T/S/T Septa, 100 PK, Part # 5182-0556 (Hewlett-Packard, Wilmington, DE)

6 Port Electrically Actuated Valve, Valco Instruments Co. Inc., PN 1384 (Alltech, Deerfield, IL)

Extractor and the Necessary Parts - DIONEX (Sunnyvale, California)

ASE™ 200 Extraction Apparatus

22-mL stainless steel extraction cells, PN 4561

Glass fiber filter, PN 47017

Cellulose filter, PN 49458

Collection vials, PN 49466

Septa for collection vial lids, PN 49464

O-rings, PN 049457

PEEK seals, PN 049455

Labware

VWR Brand Vortex Geni 2 Mixer, 115V, 60 Hz, Cat. No. 58815-178 (VWR Scientific, Boston, MA)

Biohit Proline Electronic Pipettors, Variable Volume with Tip Ejector, Vanguard, 5-100 µL Cat. No. 53495-200, 50-1000 µL Cat. No. 53495-205 and 0.10-5.0 mL Cat. No. 53495-290 (VWR Scientific, Boston, MA)

Balances - Mettler AE160 analytical and PE600 top-loading balances (Mettler Instrument Corp., Hightstown, N.J.)

Centrifuge Tubes - Kimex Brand Conical Centrifuge Tubes with Standard Taper Stopper, 13-mL capacity, Cat. No. Kimex -45176 (VWR Scientific, Boston, MA)

Miscellaneous

Filter - Non-sterile, Millex HV_{13}, 0.45 µm 13 mm Filter Unit, Cat. No. SJHV 013 NS (Millipore, Inc., Milford, MA)

3.4 Reagents

All reagents used were the same as in the original method report with the exception of water.

Water - EM Omni Solv®, HPLC-grade water, #WX0004-1, (EM Science, Gibbstown, NJ)

Oxamyl Standard: Prepared by DuPont Agricultural Products, Global Technology Division, E. I. du Pont de Nemours and Company (DPX-D1410, Lot # 376, 100.0% Purity)

Oxime Standard: Prepared by DuPont Agricultural Products, Global Technology Division, E. I. du Pont de Nemours and Company (IN-A2213, Lot # 10, 99.9% Purity)

3.5 Principles of the Analytical Method

The analytical method involved the extraction of oxamyl and oxime from soil using an ASE system. An ASE system used elevated temperature and pressure during

extraction. The samples were mixed with silica gel prior to loading into the ASE extraction cell. The ASE parameters were:

Extraction solvent:	0.01% formic acid in acetonitrile/methanol (80:20 v:v)
Static time:	5 min.
Flush volume:	100%
Purge time:	60 sec.
Extraction temperature:	50°C
Extraction pressure:	1000 psi

A 5-mL aliquot of the extract was removed. A 1-mL aliquot of 0.01% aqueous formic acid was added and the extracts were concentrated under a flow of nitrogen. The samples were then diluted to a 10-mL volume using a 0.01% aqueous formic acid solution. The samples were then syringe filtered and analyzed using LC/MS detection. The instrument used for sample analysis during the ILV was a triple quadrupole mass spectrometer operated in the LC/MS single ion mode.

3.6 Modifications, Interpretations, and Critical Steps

Analysis was performed without any method modifications.

The elution time of oxamyl and oxime from the reversed phase column was later than the time reported in the original method. The HP1100 LC system used for ILV had only minor modification made to accommodate low flow rate mixing. The LC tubing was changed from 0.007-inch i.d. to 0.005-inch i.d. In addition, a small injection loop was installed. Initially the elution time of standards was not consistent. The column equilibrium time was extended to 30 minutes to allow for a more complete equilibration of the column prior to the next injection. In some cases, the chromatographic peak shape for oxime in soil extracts was broader then the peak shape in standards. We attributed the difference in peak shape to residual solvent remaining in the extract from the sample preparation process. The variation in peak shape did not effect the performance of the method during ILV.

3.7 Instrumentation

3.7.1 Chromatography

Analysis was conducted using a gradient-elution on a reversed-phase Hewlett Packard ODS column with 3-µm diameter packing. Conditions used for the generation of the validation data presented in this report are summarized in the following table:

System:	Hewlett-Packard HP1100 HPLC
Column:	2.1 mm i.d. × 10 cm, Hewlett Packard ODS, 3 µm diameter packing, PN 79916ODS-352
Column Temperature:	30 °C
Injection Volume:	0.020 mL
Flow Rate:	0.300 mL/min

Conditions:	A: 1 mM formate in 99:1 water:methanol (v.v.) B: 99:1 methanol: water			
	Time	%A	%B	Flow (mL/Min.)
	0.0	100	0	0.300
	13.0	74	26	0.300
	13.1	10	90	0.300
	17.0	10	90	0.300
	17.1	100	0	0.300
	30.0	100	0	0.300
Oxamyl Retention Time:	~ 13.0 min			
Oxime Retention Time:	~ 9.2 min			
Total Run Time:	30 min			

A six-port electronically activated switching valve was used to direct the flow to waste prior to and following the elution of the compounds of interest. The use of this valve reduces source contamination and enables additional samples to be analyzed prior to source cleaning. The valve switching times are given in the following table.

TIME (MINUTES)	COLUMN ELUATE FLOW
0.00-5.00	Waste
5.00-15.00	MS source
15.00-30.00	Waste

3.7.2 *LC/MS Analysis*

The quantitative analysis of oxamyl and oxime was performed using a Micromass Quattro II LC/MS/MS system. Quantitative analysis was based on the integration of a single ion. The system parameters were adjusted while a solution of oxamyl and oxime was infused directly into the electrospray ion source. A summary of the experimental conditions is provided in the following table:

Micromass Quattro LC ESI-LC/MS/MS Mass Spectrometer Conditions

Analytes	Ions Monitored	Cone Voltage	Mode
Oxime	163.0± 0.1 AMU	11V	SIR
Oxamyl	237.0± 0.1 AMU	11V	SIR
Dwell Time:	0.50 seconds		
Electrospray Voltage:	4.5 kV		
Detector Voltage:	750 V		
Source Temperatures:	150 °C		
Nebulizing Gas Flow:	15 L/h		
Drying Gas Flow:	300 L/h		

A complete list of the experimental parameters is given in Appendix 2.

3.8 Calculations

3.8.1 Methods

Average Response Factor (RF_{ave}) for the set was calculated as follows:

$$RF_{ave} = \frac{(\text{Conc. A} \div \text{Area A}) + (\text{Conc. B} \div \text{Area B}) + (\text{Conc. C} \div \text{Area C}) + \ldots}{n}$$

where Conc. X and Area X are the concentration (µg/mL) and corresponding peak area (counts) for standards run with the analysis set, and n is the number of standards analyzed. The analyte concentration in fortified samples (ppm found) was calculated as follows:

$$\text{ppm Found} = \frac{(\text{Peak area}) \times (RF_{ave}) \times (\text{Final Volume}) \times (AV/VR)}{(\text{Sample Weight})}$$

AV = ASE Volume, mL recovered from the ASE extraction

VR = Volume removed from the ASE extract for analysis

ppm Found = µg/g

ppb Found = $\mu g/g \times 1000 ng/\mu g$ = ng/g

The percent recovery found was calculated as follows:

$$\%\ \text{Recovery} = \frac{(\text{ppm Found})}{(\text{Fortification level, ppm})} \times 100$$

3.8.2 Example

For a 10.0-ppb fortified oxime sample (Data Sheet Number Soil01, sample *Soil Control + 10 ppb IN-A2213 (a)* in Appendix 3), the concentration found was calculated as follows:

Average Response Factor was calculated as follows:

$$RF_{ave} = \frac{(0.0003 \mu g/mL \div 9155 AC) + (0.001 \mu g/mL \div 30305 AC) + \ldots}{6}$$

(AC ≡ Area Counts)

$RF_{ave} = 3.251e-8$ µg/mL/AC

ppm found was calculated as follows:

$$\text{ppm Found} = \frac{(59419\ \text{AC}) \times (3.251\text{e-}8\ \mu\text{g/mL/AC}) \times (10\ \text{mL}) \times (32\ \text{mL/5mL})}{(12.48\ \text{g})}$$

ppm Found = 0.0099 µg/g

(ppm values are reported to two significant figures)

ppb Found = 0.0099 µg/g x 1000 ng/µg = 9.906 ng/g

(ppb values are reported to two significant figures)

The percent recovery found was calculated as follows:

$$\%\ \text{Recovery} = \frac{(9.906\ \text{ng/g})}{(10.00\ \text{ng/g})} \text{ x } 100$$

% Recovery = 99%

(percent recoveries are rounded to the nearest whole number)

APPENDIX 4
SYNOPSIS OF COMMUNICATIONS BETWEEN JAMES J. STRY (STUDY DIRECTOR), MICHAEL R. GAGNON (DESIGNEE), AND JOHN M. BRISBIN (SPONSOR REPRESENTATIVE)

1) Protocol Step 2 – Areas Requiring Clarification

Method was clear and interpretations were not needed. However, questions were asked concerning particular steps and setup.

2) Protocol Step 2 – Questions Asked to the Sponsor Representative after reading the method

February 29, 2000

People: John Brisbin (Sponsor Representative), James Stry (Study Director)

4.2.5 Fortification Standard Preparation and Stability
Do we need to prepare standards using the successive dilution approach or can different aliquots be used.

Response: Not an issue, different aliquots are fine.

4.3.1 Descriptions and Operating Conditions - Was a split used? If so, what was the splitting ratio?

Response: A split was not used but may be added if the instrumentation would function better using a split.

4.2.8 Sample Fortification Procedure - The method instructs to weigh out 13 g of soil with a dry weight of 12.5g. Should the sample fortification be based on 13 or 12.5 grams?

Response: Fortification is based on dry weight, 12.5 grams.

4.2.9 Analyte Extraction Procedure - How many milliliters of extraction solvent will be collected from the ASE and what will be the final concentration of sample following the sample preparation procedure? Based on my calculations a 0.5-ng/mL standard will be the lowest standard required for analysis of LOQ fortifications.

Response: The extract volume will range from 25-45 mL. The final concentration will range from 2.5-1.38 ng/mL. The 0.5 ng/mL is the lowest standard required.

3) Protocol Step 3 – Calibration Curve Generation, Interference Check and Test of Reagent Substitutions

March 6, 2000

People: John Brisbin (Sponsor Representative), Tim Devine (Method Developer) and Mike Gagnon (Designee)

Background:
After injection of several standards, a good standard curve was achieved (0.999 RSQ). Retention time is about 3 minutes later than expected and is not very stable. It is possible the HP 1100 LC system used to develop the method was modified in some way to cause the shorter retention time. I spoke with Tim and John about these particular issues.

Questions Asked:
Is the HP 1100 LC used to develop the method different from the HP 1100 LC used to validate the method in any way? If so, would the modifications account for the difference in retention times?

Response:
Yes, the LC used to develop the method has been re-plumbed with smaller ID tubing than that used in the LC to validate the method. There was a smaller mixing chamber installed (81 µL vs 480 µL) and there was also a smaller sample loop installed. It was also noted that the tubing used to transfer the analyte from the LC into the MS was kept to a minimum.

The LC used for method validation will be re-plumbed with the smaller ID tubing, the smaller injection loop will be installed and the tubing, which transfers the sample from the LC into the MS, will be minimized. All this should decrease the retention time somewhat. The smaller mixing chamber would probably make a big difference but is not available.

After making these modifications, a series of standards will be analyzed. If an acceptable standard curve is generated, we will proceed with the validation of the method, making note of the later retention time due to the larger volume in the mixing chamber.